

WHAT IS CLAIMED IS:

1. A simple and accurate method for assay of a single-stranded RNA containing a specific nucleic acids sequence in a sample at almost constant temperature by using at least the following reagents (A) to (I), which comprises a step of adding the reagents (A) to (I) one by one (in any order), in combinations of at least two or all at once and

10 a step of measuring a fluorescent signal in the presence of the reagent (I) at least once after addition of at least the reagents (A) to (H);

(A) a first single-stranded oligonucleic acid complementary to a sequence neighboring the 5' end of the specific nucleic acids sequence in the single-stranded RNA,

15 (B) a second single-stranded oligo DNA complementary to a 3'-end sequence within the specific nucleic acids sequence,

(C) an RNA-dependent DNA polymerase,

20 (D) deoxyribonucleoside triphosphates,

(E) a third single-stranded oligo DNA having (1) a promoter sequence for a DNA-dependent RNA polymerase, (2) an enhancer sequence for the promoter and (3) a 5'-end sequence within the specific nucleic acids sequence, in this order from the 5' end,

25 (F) a DNA-dependent DNA polymerase,

(G) a DNA-dependent RNA polymerase,

(H) ribonucleoside triphosphates, and

(I) a fourth single-stranded oligo DNA complementary to the specific nucleic acids sequence which is labeled so that it gives off a measurable fluorescent signal on

5 hybridization with a nucleic acid containing the specific nucleic acids sequence.

2. The method according to Claim 1, wherein the temperature is selected from the range of from 35 to 60°C.

3. The method according to Claim 1, wherein the first

10 oligonucleic acid as the reagent (A) is a DNA, and the method further comprises a step of adding an RNaseH and a subsequent step of deactivating the RNaseH by heating or by addition of an inhibitor prior to addition of the reagent (B).

15 4. The method according to Claim 3, wherein addition of the reagent (A) is followed by simultaneous addition of the reagents (B) to (H), and further by addition of the reagent (I).

5. The method according to Claim 3, wherein addition of

20 the reagent (A) is followed by simultaneous addition of the reagents (B) to (I).

6. The method according to Claim 1, wherein the first oligonucleic acid as the reagent (A) is a ribozyme or a

DNAzyme.

25 7. The method according to Claim 1, which further uses dimethyl sulfoxide and/or an enzyme which degrades RNA in a DNA-RNA double strand.

8. The method according to ~~Claim 7~~, which uses dimethyl sulfoxide at a concentration of from 5 to 20%.

9. The method according to ~~Claim 7~~, wherein the enzyme which degrades RNA in a DNA-RNA double strand is the RNA-dependent DNA polymerase as the reagent (C).

10. The method according to ~~Claim 1~~, wherein an enzyme having both an RNA-dependent DNA polymerase activity and a DNA-dependent DNA polymerase activity is used as the reagents (C) and (F) to virtually omit addition of the reagent (C) or the reagent (F).

11. The method according to ~~Claim 10~~, wherein the enzyme is avian myoblastome virus polymerase.

12. The method according to ~~Claim 2~~, wherein the second and third oligo DNAs as the reagents (B) and (E) are used at concentrations of from 0.02 to 1 μ M.

13. The method according to ~~Claim 1~~, wherein the DNA-dependent RNA polymerase as the reagent (G) is at least one enzyme selected from the group consisting of phage SP6 polymerase, phage T3 polymerase and phage T7 polymerase.

14. The method according to ~~Claim 1~~, wherein the fourth oligo DNA as the reagent (I) is a DNA which is linked to a fluorescent intercalative dye so that the fluorescent intercalative dye changes its fluorescence characteristic on hybridization of the DNA with another nucleic acid by intercalating into the resulting double strand.

15. The method according to ~~Claim 1~~ or 14, wherein the

fourth oligo DNA as the reagent (I) is a DNA which has a 3'-end sequence uncomplementary to the specific nucleic acids sequence or has a modified 3' end.

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5 16. The method according to Claim 1, which further comprises a step of detecting or quantifying the single-stranded RNA in the sample based on the measured fluorescent signal or change in the measured fluorescent signal.

10 17. The method according to Claim 1, wherein all the reagents are chloride-free.

18. The method according to Claim 1, which further uses an acetate.

15 19. The method according to Claim 18, wherein the acetate is magnesium acetate at a concentration of from 5 to 20 mM or potassium acetate at a concentration of from 50 to 200 mM.

20 20. The method according to Claim 1, which further uses sorbitol.

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21. A simple method for producing a nucleic acid having a 20 specific nucleic acids sequence at almost constant temperature by using at least the following reagents (A) to (H), which comprises a step of adding the reagents (A) to (G) one by one (in any order), in combinations of at least two or all at once to a single-stranded DNA having 25 (1) a promoter sequence for a DNA-dependent RNA polymerase, (2) an enhancer sequence for the promoter and (3) the specific nucleic acids sequence, in this order

from the 5' end or to a double-stranded DNA consisting of the single-stranded DNA and a complementary DNA strand and a step of measuring a fluorescent signal from the reagent (H) at least once after addition of at least the 5 reagents (A) to (G);

- (A) a single-stranded oligo DNA complementary to a 3'-end sequence within the specific nucleic acids sequence,
- (B) an RNA-dependent DNA polymerase,
- (C) a DNA-dependent DNA polymerase,
- 10 (D) deoxyribonucleoside triphosphates,
- (E) a DNA-dependent RNA polymerase,
- (F) ribonucleoside triphosphates,
- (G) a single-stranded DNA having (1) a promoter sequence for a DNA-dependent RNA polymerase, (2) an enhancer 15 sequence for the promoter and (3) a 5'-end sequence within the specific nucleic acids sequence, in this order from the 5' end,
- (H) a fourth single-stranded labeled oligo DNA complementary to the specific nucleic acids sequence
- 20 which gives a measurable fluorescent signal on hybridization with a nucleic acid containing the specific nucleic acids sequence.

22. The method for producing a single-stranded RNA having a specific nucleic acids sequence according to Claim 21, 25 wherein a DNase is added when the measured fluorescent signal or change in the measured fluorescent signal indicates production of a predetermined amount of the

specific nucleic acids sequence.

23. The method for producing a double-stranded DNA consisting of a DNA strand having a specific nucleic acids sequence and a complementary DNA strand according 5 to Claim 21, wherein an RNase is added when the measured fluorescent signal or change in the measured fluorescent signal indicates production of a predetermined amount of the specific nucleic acids sequence.

24. A reagent set for performing the method according to 10 Claim 1 or 21, which comprises at least a first reagent containing the first single-stranded oligonucleic acid, a second reagent containing tris-acetate, magnesium acetate, potassium acetate, sorbitol and dimethyl 15 sulfoxide, a third reagent containing dithiothreitol, deoxyribonucleoside triphosphates, ribonucleoside triphosphates, bovine serum albumin, the second single-stranded oligo DNA and the third single-stranded oligo 20 DNA, a fourth reagent containing an RNA-dependent DNA polymerase, a DNA-dependent DNA polymerase, a DNA-dependent RNA polymerase and an RNase inhibitor and a fifth reagent containing the fourth single-stranded 25 oligo DNA.

25. A reagent set for performing the method according to Claim 1 or 21, which comprises at least

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a first reagent containing the first single-stranded oligonucleic acid,

a second reagent containing tris-acetate, magnesium acetate, potassium acetate, sorbitol and dimethyl sulfoxide,

5 a third reagent containing dithiothreitol, deoxyribonucleoside triphosphates, ribonucleoside triphosphates, bovine serum albumin, the second single-stranded oligo DNA, the third single-stranded oligo DNA and the fourth single-stranded oligo DNA and

10 a fourth reagent containing an RNA-dependent DNA polymerase, a DNA-dependent DNA polymerase, a DNA-dependent RNA polymerase and an RNase inhibitor.

26. A reagent set for performing the method according to

15 Claim 1 or 21, which comprises at least

a first reagent containing the first single-stranded oligonucleic acid,

a second reagent containing tris-acetate, magnesium acetate, potassium acetate, sorbitol and dimethyl sulfoxide,

20 a third reagent containing dithiothreitol, deoxyribonucleoside triphosphates, ribonucleoside triphosphates, bovine serum albumin, the second single-stranded oligo DNA and the third single-stranded oligo DNA,

25 a fourth reagent containing the fourth single-stranded oligo DNA, an RNA-dependent DNA polymerase, a DNA-

dependent DNA polymerase, a DNA-dependent RNA polymerase and an RNase inhibitor.

27. A reagent for performing the method according to
Claim 1 or 21, which comprises at least the first single-
5 stranded oligonucleic acid, the second single-stranded
oligo DNA, the third single-stranded oligo DNA, the
fourth single-stranded oligo DNA, an RNA-dependent DNA
polymerase, a DNA-dependent DNA polymerase, a DNA-
dependent RNA polymerase, deoxyribonucleoside
10 triphosphates, ribonucleoside triphosphates, tris-acetate,
magnesium acetate, potassium acetate, sorbitol, dimethyl
sulfoxide, dithiothreitol, bovine serum albumin and an
RNase inhibitor.

28. The reagent set or reagent according to any one of
15 Claims 24 to 27, wherein an enzyme having both an RNA-
dependent DNA polymerase activity and a DNA-dependent DNA
polymerase activity is used at least as the RNA-dependent
DNA polymerase and as the DNA-dependent DNA polymerase.

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